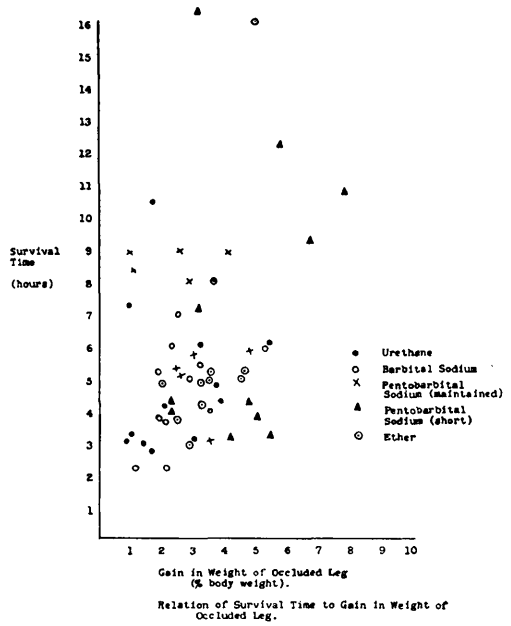

FIG. 1.

Relation of survival time to gain in weight of Occluded Leg.

little influence on the outcome as judged by the results of the 2 pentobarbital series. This circumstance may have been due to 2 factors: the long-lasting effect of the initial injection of pentobarbital sodium and the fact that additional anesthetic was not required when the mean arterial blood pressure had fallen to about 70 mm Hg.

The wide variation in the values for the loss of fluid into the occluded leg (1.0-7.9%) indicates that although reduced blood volume may be a contributing factor, it is probably not the only factor in this type of experimental shock. This is also supported by the lack of correlation between length of survival time and weight of the occluded leg (Fig. 1). The closest approach to a correlation is perhaps suggested in the ether series.

Summary. In this laboratory the method of venous occlusion with several types of anesthetics has consistently produced experimental shock characterized by hemoconcentration, circulatory failure and death. Although oligemia may be a contributing factor, it does not appear to be the only factor in this type of experimental shock. From the point of view of survival time there is little choice between short and maintained anesthesia. Using the same criterion, the anesthetic agents of preference are pentobarbital sodium, and ether.

13981 P

Crystalline Trypsin-Inhibitor and Blood-Clotting.

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Through the courtesy of Dr. T. E. Weichselbaum, Washington University, St. Louis, we were supplied with 10 mg of pure *crystalline* trypsin-inhibitor (1 mg $\equiv 4.0 \times 10^{-2}$ $[T.U.]_{Hb}$), a polypeptide which was originally isolated from pancreas by Northrop and Kunitz.[1] The sample, dissolved in 10 cc physiological saline, was brought from pH = 5.1 to pH = 7.2 with a trace of n/10 NaOH (= inhib.). The other clotting reagents and test methods have been described in previous publications,[2] the dialyzed crude albumin (= alb.) being prepared from *rabbit* plasma.

I. *Inhibition of clotting of recalcified citrated plasma (rabbit):* Whole plasma and several saline dilutions showed unequivocal retardation of clotting on recalcification in the presence of inhib., as compared to controls; *e.g.*, clotting-time of plasma (1:2 dilution) on adding $CaCl_2$: (a) control (with saline) = 3′ 20″; (b) with inhib. = 7′ 05″.

[1] Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1936, **19**, 991.

[2] Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

TABLE I.

Effect of Crystalline Trypsin-Inhibitor (inhib.) and Crude Plasma Albumin (alb.) on Conversion of Prothrombin to Thrombin. Temp. = 21°C, pH = 7.2. Clotting-times, in Seconds, for 0.5 cc Thrombic Mixture (Prothrombin + Ca + Thromboplastin + Inhibitor) *plus* 1.0 cc Fibrinogen.

Exp.	"Inhibitor"	Incubation period				
		2½ min	5 min	10 min	20 min	30 min.
1 a.	none (control)	—	200″	70″	42″	35″*
1 b.	alb.	—	129″	36″	22″	21″
1 c.	alb. + inhib.	—	147″	62″	44″	45″
2 a.	none (control)	25″	16″	16″	—	—
2 d.	inhib.	38″	22″	22″	—	—

* Optimum (checked at 60′ and 90′)

II. *Inhibition of thrombic clotting of fibrinogen:* A 1:100 dilution of Parfentjev's[3] "rabbit clotting globulin" (Lederle Lab.) proved an excellent and stable thrombin (T). The following clotting-times (C.T.) were determined at room temperature (21 C) and pH = 7.2, the thrombin, in each case, being mixed with the cited agents for 10 min prior to the addition of the fibrinogen (F):

1. 1 cc F + 0.5 cc T + 0.5 cc saline ..... C.T. = 23 sec.
2. 1 cc F + 0.5 cc T + 0.25 cc saline + 0.25 cc inhib. ..... C.T. = 24 sec.
3. 1 cc F + 0.5 cc T + 0.25 cc saline + 0.25 cc alb. ..... C.T. = 48 sec.
4. 1 cc F + 0.5 cc T + 0.25 cc inhib. + 0.25 cc alb. ..... C.T. = 2760 sec.

No. 4 shows a definite antithrombic effect, as compared with the absence of any direct action of the inhibitor, alone, on the thrombin-fibrinogen interaction (No. 2).

III. *Effect of inhibitor on thrombin formation:* In the routine method[2] for quantitating the activation of prothrombin to thrombin, 0.5 cc of thrombic mixture is sampled after the cited incubation periods and clotting-time noted on adding sample to 1.0 cc test fibrinogen. Thrombic mixtures have a uniform 5 cc volume, made up with saline, as necessary. Each contains 4 cc of Howell-type prothrombin *plus* "activators" and any inhibitors it is desired to study. Two separate experiments are included in Table I, each control (a) employing, as "activators," 0.25 cc N/10 $CaCl_2$ + 0.25 cc dil. brain thromboplastin. In addition, the inhibitory tests have (b) 0.25 cc alb., (c) 0.25 cc alb. + 0.25 cc inhib. or (d) 0.25 cc inhib., respectively. The optimum (shortest) clotting-time is a relative measure of thrombin yield, for each particular experiment.

The few seconds prolongation of optimal clotting-time in the presence of inhib. is significant (*cf.* II) and resembles data previously reported[4] for heparin, except that it is very weak. There is no evidence that the crude albumin supplies a first-phase cofactor, but no significant conclusions as to the possible extent of first-phase inhibitions by the crystalline polypeptide could be reached for at least three reasons, viz. 1. insufficiency of inhib., 2. interfering effect of the incidental (previously noted[4]) thromboplastic effect of the crude albumin (which is probably associated with the presence of serum-tryptase), 3. considerations regarding a "time-factor" (v. infra).

IV. *Antifibrinolytic effect:* The use of thymol-saline kept the tubes free from bacterial growth and it was noted that, whereas the controls (with saline) showed "spontaneous" fibrinolysis complete on the 3rd day, such fibrinolysis was incomplete until the 4th day in the tubes containing albumin (alone) and entirely absent, even at the end of a week, in the tubes containing crystalline inhibitor (with or without alb.). This confirms our previously expressed view that the natural "fibrinolysin," so frequently present, in very small traces, in fibrinogen and other plasma protein products, is none other than the same *serum-tryptase* to which we ascribe the important thromboplastic and variable

[3] Parfentjev, I. A., *Am. J. Med. Sci.*, 1941, **202**, 578.

[4] Ferguson, J. H., *Am. J. Physiol.*, 1941, **134**, 47.

incidental (*e.g.* thrombinolytic or "progressive" antithrombic) effects.

Summary. The data, as a whole, clearly indicate that the crystalline polypeptide tested has a weak inhibitory action on blood-clotting systems. There is good evidence for an antifibrinolytic as well as for antiprothrombic and antithrombic effects. A co-factor seems to be necessary, at least for the last, and it can be supplied in crude plasma albumin. The similarities to the actions of (small quantities of) heparin[4] are striking and the minor discrepancies may be due to such experimental variables as impurity of the albumin, inadequate amounts of inhibitor, and other experimental conditions. In contrast to the "immediate" character of typical heparin effects,[4] there is, in the present experiments, a definite suggestion of a *time-factor* required for the full development of the antithrombic and other inhibitory effects. This may very well explain part of the difficulty encountered in demonstrating more convincingly the first-phase inhibition by the crystalline polypeptide.

These experiments are preliminary and require confirmation with larger amounts of polypeptide and extended study of the question as to optimal conditions for the inhibitory effects. Nevertheless, they are highly suggestive and fit in with a new blood-clotting theory,[5] which revolves around the dominant role of serum-tryptase and its inhibitors in natural blood-coagulation systems.

[5] Ferguson, J. H., *Science*, in press.

13982

A Simple Method for Determining Effective Renal Blood Flow and Tubular Excretory Mass in Man.*

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Quantitative methods for the determination of the tubular excretory mass and the rate of blood flow through the active renal tissue by diodrast clearance have been developed by Smith and his associates[1] and have been used in recent years almost entirely for the purpose of research. We have suggested recently[2-4] that the determination of the effective renal blood flow in patients with arterial hypertension might furnish not only interesting data on the pathogenesis of the disease, but also information of considerable value for the prognosis and selection of patients to be submitted to surgical treatment.

To confirm this possibility it became necessary to find a simple method suitable for the routine clinical study of a large number of patients. The methods of Smith and his associates are not practical as they require continuous intravenous infusion, inlying catheter, the full attention of a physician and a technician for several hours, and a large number of chemical determinations.

To our knowledge, Findley and White[5] made the only attempt to simplify the determination of effective renal blood flow, by the use of a single subcutaneous injection of diodrast. They measured effective renal

* This work was made possible by a grant from the Aaron Mendelson Memorial Trust Fund.

† Research Fellow.

[1] Smith, H. W., Goldring, W., and Chasis, H., *J. Clin. Invest.*, 1938, **17**, 263.

[2] Foà, P. P., Woods, W. W., Peet, M. M., and Foà, N. L., *Arch. Int. Med.*, 1942, **69**, 822.

[3] Foà, P. P., Woods, W. W., Peet, M. M., and Foà, N. L., *Arch. Int. Med.*, in press.

[4] Foà, M. M., Woods, W. W., Peet, M. M., and Foà, N. L., *Univ. Hosp. Bull.*, Ann Arbor, 1942, **8**, 9.

[5] Findley, T., and White, H. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 623.